

AD A 051 779

UNCLASSIFIED

UNLIMITED  
DISTRIBUTION

**DRES**

## SUFFIELD TECHNICAL PAPER

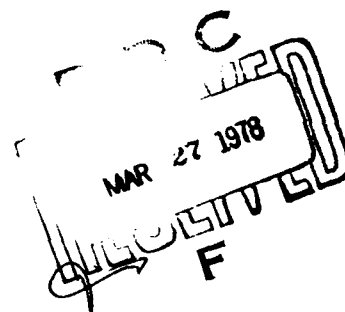
NO. 487

EFFICACY OF PRO-PAM  
(N-METHYL-1,6-DIHYDROPYRIDINE-2-CARBALDOXIME HYDROCHLORIDE  
AS A TREATMENT FOR ORGANOPHOSPHATE POISONING (U)

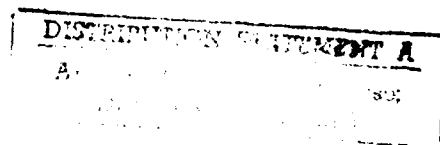
AD INU.  
DDC FILE COPY

by

J.G. Clement



PROJECT NO. 13016



February 1978



DEFENCE RESEARCH ESTABLISHMENT SUFFIELD : RALSTON : ALBERTA

### WARNING

The use of this information is permitted subject to recognition  
of proprietary and patent rights.

UNCLASSIFIED

UNLIMITED  
DISTRIBUTION

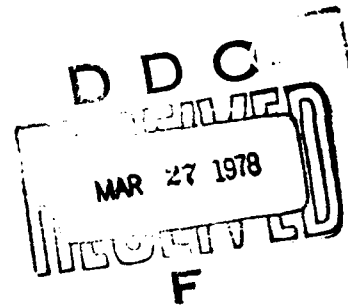
DEFENCE RESEARCH ESTABLISHMENT SUFFIELD  
RALSTON ALBERTA

SUFFIELD TECHNICAL PAPER NO. 487

EFFICACY OF PRO-PAM  
(N-METHYL-1,6-DIHYDROPYRIDINE-2-CARBALDOXIME HYDROCHLORIDE)  
AS A TREATMENT FOR ORGANOPHOSPHATE POISONING (U)

by

J.G. Clement



PROJECT NO. 13D16

**WARNING**

The use of this information is permitted subject to recognition  
of proprietary and patent rights.

UNCLASSIFIED

#### ACKNOWLEDGEMENTS

I wish to thank Dr. N. Bodor, InterX Corporation and Miss Janet Rose, Organic Chemistry Section, DRES for their generous supplies of Pro-PAM and Hoffman La Roche for supplying the pyridostigmine used in this study. The technical assistance of Mr. H. Copeman is acknowledged.

ACCESSION NO.	
NHS	<input checked="" type="checkbox"/>
DOC	<input type="checkbox"/>
REMARKS	<input type="checkbox"/>
U.S. DEPT. OF HEALTH	
DISPOSITION AND ACQUISITION	
A	

UNCLASSIFIED

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD  
RALSTON ALBERTA

SUFFIELD TECHNICAL PAPER NO. 487

EFFICACY OF PRO-PAM  
(N-METHYL-1,6-DIHYDROPYRIDINE-2-CARBALDOXIME HYDROCHLORIDE)  
AS A TREATMENT FOR ORGANOPHOSPHATE POISONING (U)

by

J.G. Clement

ABSTRACT

The efficacy of Pro-PAM (N-methyl-1,6-dihydropyridine-2-carbaldoxime hydrochloride) was compared to pyridine-2-aldoxime chloride (PAM) as a treatment for organophosphate poisoning in mice and guinea pigs. Pro-PAM was generally less toxic than PAM in mice and pH of the vehicle did not appear to alter the toxicity of Pro-PAM. Pro-PAM alone and combined with atropine improved the protective ratio (PR) of DFP and sarin in mice, whereas it improved very slightly the PR of soman in guinea pigs. The PR obtained with Pro-PAM combined with carbamate prophylaxis and atropine therapy versus soman poisoning was inferior to that obtained with PAM. In most cases Pro-PAM and atropine prophylaxis produced higher brain acetylcholinesterase (AChE) levels than PAM and atropine, however there was no correlation between brain AChE levels and survival. The significance of this lack of correlation is discussed. Pro-PAM was not a significant improvement over PAM with regards to therapy of organophosphorus poisoning.

(U)

UNCLASSIFIED

UNCLASSIFIED

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD  
RALSTON ALBERTA

SUFFIELD TECHNICAL PAPER NO. 487

EFFICACY OF PRO-PAM  
(N-METHYL-1,6-DIHYDROPYRIDINE-2-CARBALDOXIME HYDROCHLORIDE)  
AS A TREATMENT FOR ORGANOPHOSPHATE POISONING (U)

by

J.G. Clement

INTRODUCTION

The use of atropine as well as oximes is essential for efficient therapy against poisoning by various organophosphorus compounds (Johnson and Stewart, 1970; Natoff and Reiff, 1970). However, one of the problems in treatment of organophosphate poisoning is getting the reactivator of acetylcholinesterase (AChE) (e.g. pyridine-2-aldoxime (PAM)) across the blood brain barrier (BBB). It is commonly thought that reactivation of brain AChE and protection of the post-synaptic receptors will allow the central respiratory centre to maintain its normal function, thus accounting for the therapeutic effectiveness of the atropine, oxime treatment.

Various workers have found that systemic administration of PAM does not reactivate phosphorylated AChE in the brain (Kewitz and Nachmansohn, 1957; Hobbiger, 1957; O'Leary et al., 1959) even when given in doses which produce a marked reactivation at peripheral sites (Edery and Shatzberg-Porath, 1959; Fleisher et al., 1960; Milosevic et al., 1961). However, other workers have found evidence suggesting that PAM may have a central action: PAM reactivated brain AChE (Aarseth and

UNCLASSIFIED

Barstad, 1968; Rosenberg, 1960); had a definite effect on EEG of rabbits exposed to sarin (Rosenberg, 1960); induced rapid recovery of consciousness; and had anticonvulsant effects in people poisoned by parathion (Namba and Hiraki, 1958; Karlog et al., 1958; Funches, 1960; Schuchter et al., 1960). These effects may be due to the fact that the BBB is not complete, i.e. certain areas of the brain (choroid plexus, area postrema, neurohypophysis, fornix) are more accessible to quaternary ammonium compounds than others (Ellin and Wills, 1964; Rosenberg, 1960; Wilson et al., 1962; Firemark et al., 1964; Milosevic and Andjelhovic, 1966; Hobbiger and Vojvodić, 1966).

Bodor et al. (1976) and Shek et al. (1976a, 1976b) recently described the synthesis, metabolism and disposition of N-methyl-1,6-dihydropyridine-2-carbaldoxime hydrochloride (Pro-PAM), a pro-drug<sup>1</sup> of PAM. The pKa of Pro-PAM was 6.32; thus at physiological pH approximately 90% of the drug would be in the unionized form. Administration of Pro-PAM resulted in higher brain levels of PAM and more reactivation of phosphorylated AChE compared to animals receiving PAM (Shek et al., 1976b).

The purpose of this study was to evaluate the effectiveness of Pro-PAM, as compared to PAM alone and combined with atropine, in protecting mice and guinea pigs against the toxic effects of various organophosphate anticholinesterases.

## METHODS

### Toxicological Studies

In all experiments male mice (Charles River - CDA) (25-30 g) and female guinea pigs (Hartley - Albino) (300-400 g) were used. The

---

<sup>1</sup>"Pro-drug denotes a derivative of a known and proven drug, which derivative, due to its improved physiochemical properties, increases the bioavailability of the proven drug and which derivative is transformed by an enzymatic or chemical process into the proven drug before reaching and/or at the site(s) of action."  
(Bodor et al., 1976)

prophylaxis was administered im, in the hind limb, 10 minutes prior to receiving the organophosphorus anticholinesterase sc, in the scruff of the neck. In preliminary experiments in mice, it was found that the 10 minute pretreatment time was optimum. Longer pretreatment times resulted in lower protective ratios. The prophylaxis was administered in either a pH 3.0 citrate buffer or a saline solution at pH 7.0. The injection volumes were 1.0% and 0.1% of body weight in mice and guinea pigs, respectively. The LD<sub>50</sub> was calculated by means of probit analysis and results were expressed as a protective ratio (PR equals the ratio of LD<sub>50</sub> of the toxic substance in treated animals to that in unprotected animals). The drugs were evaluated as a prophylaxis since this would ensure that they had reached adequate blood levels before administration of the organophosphorus anticholinesterase, thus better assessing the treatment's ability to protect the animal.

#### Brain AChE Determinations

Total brain AChE activity was determined in control animals and in those surviving the various treatments. Doses of organophosphates were used which in preliminary studies depressed brain AChE values without producing any mortality. Thirty minutes after receiving organophosphorus anticholinesterase, the animals were sacrificed by decapitation and exsanguination. The brain was removed, rinsed in cold saline, blotted dry and weighed. It was homogenized in the cold in a sufficient amount of 0.9% saline to give a final solution of 100 mg brain tissue/ml of saline.

Brain AChE activity was determined immediately by the titrimetric procedure as described by Jensen-Holm et al. (1959). AChE activity was expressed as the number of  $\mu$ moles of ACh hydrolysed/g of brain tissue/minute.

The student "t" test was used to analyse all the data; a difference of  $p \leq 0.05$  was considered significant.

#### MATERIALS

Soman, sarin and PAM-Cl were obtained from the Organic Chemistry

Section, Defence Research Establishment Suffield, Alberta, Canada. Diisopropylfluorophosphate (DFP) was obtained from Aldrich Chemical Company.

Aqueous stock solutions of soman, sarin and DFP were prepared immediately prior to injection and diluted to the required concentration with 0.9% saline. At higher concentrations, DFP would not dissolve in water, therefore a stock solution was prepared with ethanol as the solvent. This stock solution was then diluted 1:10 to give the required concentration for injection. Control injections were similarly prepared with the organophosphate anticholinesterase being absent.

Pro-PAM was prepared according to the method of Bodor et al. (1976) by the Organic Chemistry Section. Infra-red spectrum and elemental analysis of our material were identical to that obtained by Bodor (personal communication). Immediately prior to injection, Pro-PAM was dissolved in pH 3.0 citrate buffer since this solution was found to be more stable than an unbuffered aqueous solution or a solution at a higher pH (Bodor et al., 1976; Bodor, personal communication; Clement, unpublished results).

## RESULTS

The data in table 1 demonstrate the effect of route of administration of Pro-PAM and PAM in mice. Following ip administration, the LD<sub>50</sub>s of the two drugs were very similar, whereas Pro-PAM was more toxic by im administration and less toxic than PAM following iv administration. The pH of the injected solution did not have any significant effect on the LD<sub>50</sub> of Pro-PAM following im administration.

Following im administration of a lethal dose of Pro-PAM, the mice became lethargic and prostrate within 8-12 minutes following injection. Tremor became visible, with respiratory arrest occurring 20-25 minutes post injection. The urine in Pro-PAM-treated animals was orange-amber coloured. This urine colour was probably caused by Pro-PAM being excreted in the urine. Due to the small amounts of urine, no attempt was made to isolate and identify Pro-PAM chemically. In guinea pigs,



the LD<sub>50</sub> of Pro-PAM following im administration lies between 40 and 80 mg/kg. (At 40 mg/kg 0/5 deaths and at 80 mg/kg 5/5 died.) Death was most probably due to anoxia caused by respiratory failure, since the heart was still beating following cessation of breathing. Cholinergic stimulation was not obvious, although a few animals exhibited lacrimation but not excess salivation.

The results in table 2 demonstrate that Pro-PAM alone or combined with atropine as a prophylaxis was more effective than PAM against DFP poisoning while having no significant effect against soman or sarin in mice. In guinea pigs (table 3), Pro-PAM + atropine increased the protective ratio of soman but not sarin over that obtained with PAM + atropine. However, the inclusion of Pro-PAM in a carbamate prophylaxis regimen was less effective than that obtained with PAM (table 4). It was noted that the survivors were not incapacitated in any obvious way, however they did not consume as much water as normal untreated guinea pigs.

The results in table 5 indicate that in mice the treatment of Pro-PAM + atropine resulted in significant differences ( $p < 0.01$ ) in the activity of whole brain AChE compared to animals similarly treated with PAM + atropine. It is interesting that only in the case of DFP did Pro-PAM + atropine prophylaxis produce a sizeable increase in the protective ratio as compared to that following PAM + atropine (table 2). In guinea pigs, Pro-PAM + atropine produced a significant increase in brain AChE activity in sarin treated animals, whereas in soman treated animals Pro-PAM + atropine did not (table 6).

The results in table 7 are brain AChE values from animals exposed to sarin. In animals killed with sarin (125  $\mu$ g/kg) brain AChE activity was still detected, while at higher doses of sarin (300  $\mu$ g/kg) no brain AChE activity was found. It is evident that there is no relationship between the level of brain AChE activity and survival.

#### DISCUSSION

Pro-PAM and PAM Cl had similar LD<sub>50</sub>s when administered ip,

whereas Pro-PAM was more toxic than PAM Cl when administered im or iv. Also, Pro-PAM was more toxic when administered im than iv. This apparent anomaly was probably due to the altered distribution and bio-availability of PAM following Pro-PAM administration. Shek et al. (1976a) found that Pro-PAM was converted to PAM in vivo and that this resulted in a longer biological half-life and a different pattern of distribution of PAM.

Pro-PAM was evaluated as a prophylaxis alone and combined with atropine against poisoning by various organophosphate anticholinesterases. The prophylactic administration was to ensure that the oxime was at the site of action prior to administration of organophosphate anticholinesterase. By using this approach, differences in the efficacy of Pro-PAM vs PAM would not be due to differences in absorption or distribution but would be due to effect(s) at the active site(s). Pro-PAM plus atropine prophylaxis increased the protective ratio of DFP in mice and soman in guinea pigs. Given prophylactically, Pro-PAM alone increased slightly the protective ratio against DFP poisoning in mice.

Oxime given partly as prophylaxis and partly as therapy appears to be the most effective treatment (Gordon and Leadbeater, 1977). Gordon et al. (1977) demonstrated that oxime plus carbamate prophylaxis combined with oxime plus atropine therapy greatly increased the protective ratio in guinea pigs against extremely toxic organophosphorus poisons. Pro-PAM was evaluated in the above scheme, however, the results in table 4 demonstrate that PAM was superior to Pro-PAM.

Previous investigators have reported that PAM does not reactivate in vivo brain AChE but therapeutic doses in vitro will reactivate brain AChE (Ellin and Wills, 1964). Pro-PAM + atropine in almost all cases examined caused a significant increase in brain AChE activity in mice and guinea pigs when compared to animals treated with PAM + atropine. The increased brain levels of PAM following Pro-PAM administration (Shek et al., 1976b) were probably responsible for the reactivation of brain AChE observed. Since the oxime was probably present in the CNS, it is possible that soman-inhibited AChE was reactivated before

it aged and/or less soman was present to inhibit the AChE due to a direct action of the oxime and organophosphate as proposed by Heilbronn and Tolagen (1965). The variable reactivation of brain AChE in animals treated with soman may be due to species differences with regards to penetration of the oxime to the brain.

The interesting point to emerge from this study was a lack of correlation between reactivation of brain AChE and an increase in the protective ratio. Similarly, Hobbiger and Vojvodić (1966) found no correlation between the reactivation of brain AChE and the antidotal actions of PAM combined with atropine against organophosphorus poisoning. They concluded that the antidotal properties of pyridinium aldoxime depend upon reactivation of AChE at peripheral sites. There appear to be differences in AChE in various areas with respect to inhibition by organophosphates and reactivation by oximes. Jakl and Tabuch (1971) found that soman inhibited brain AChE more than it inhibited diaphragm AChE. Mayer and Michalek (1971) found high concentrations of toxogonin in tissues where little or no reactivation of AChE had taken place. They stated that "penetration of obidoxime into a tissue is a necessary but not sufficient condition for ChE reactivation". Similarly, Adams et al. (1976) found that spontaneous recovery of central respiratory functions following poisoning with soman or sarin was possible without any noticeable reactivation of AChE activity.

Organophosphate poisoning is undoubtedly the result of inhibition of AChE, however the relationship between inhibition of brain AChE and the other neurochemical changes which occur in the ensuing death remains to be determined.

REFERENCES

- Aarseth, P. and Barstad, J.A.B. (1968) Blood Brain Barrier Permeability in Various Parts of the Central Nervous System. Arch. Int. Pharmacodyn. Therap. 176, 434-442.
- Adams, G.K., Yamamura, H.I. and O'Leary, J.F. (1976) Recovery of Central Respiratory Function Following Anticholinesterase Intoxication. Eur. J. Pharmacol. 38, 101-112.
- Bodor, N., Shek, E. and Higuchi, T. (1976) Improved Delivery Through Biological Membranes I. Synthesis and Properties of 1-Methyl-1, 6-Dihydropyridine-2-Carbaldoxime, A Pro-Drug of N-Methylpyridinium-2-Carbaldoxime Chloride. J. Med. Chem. 19, 102-107.
- Edery, H. and Schatzberg-Porath, G. (1959) Prophylactic and Therapeutic Effects of Pyridine-2-Aldoxime Methiodide and Diacetyl Monoxime Against Poisoning by Organophosphorus Compounds. Arch. Int. Pharmacodyn. Therap. 121, 104-109.
- Ellin, R.J. and Wills, J.H. (1964) Oximes Antagonistic to Inhibitors of Cholinesterase. J. Pharm. Sci. 53, 995-1007.
- Firemark, H., Barlow, C.F. and Roth, L.J. (1964) The Penetration of 2-PAM- $C^{14}$  into Brain and the Effect of Cholinesterase Inhibitors on Its Transport. J. Pharmacol. Exp. Therap. 145, 252-265.
- Fleisher, J.H., Hansa, J., Killos, R.J. and Harrison, C.S. (1960) Effects of 1,1'-Trimethylene bis(4-Formylpyridinium Bromide) Dioxime (TMB-4) on Cholinesterase Activity and Neuromuscular Block Following Poisoning with Sarin and DFP. J. Pharmacol. Exp. Therap. 130, 461-468.
- Funches, A.J. (1960) Treatment of Severe Poisoning with 2-Pyridinium Aldoxime Methiodide (2-PAM) Report of a Case. Arch. Environ. Health 1, 404-406.
- Gordon, J.J. and Leadbeater, L. (1977) The Prophylactic Use of P<sub>2</sub>S (1-Methyl,2-Hydroxyiminomethyl-Pyridinium Methanesulfonate) in the Treatment of Organophosphate Poisoning. Toxicol. Appl. Pharmacol. (in press)
- Gordon, J.J., Leadbeater, L. and Maidment, M.P. (1977) The Protection of Animals Against Organophosphate Poisoning by Pretreatment with a Carbamate. Toxicol. Appl. Pharmacol. (in press)
- Heilbronn, E. and Tolagen, B. (1965) Toxogonin in Sarin, Soman and Tabun Poisoning. Toxicol. Appl. Pharmacol. 14, 73-77.

REFERENCES (Con't)

- Hobbiger, F. (1957) Protection Against the Lethal Effects of Organo-Phosphates by Pyridine-2 Aldoxime Methiodide (PAM). Brit. J. Pharmacol. 12, 438-446.
- Hobbiger, F. and Vojvodić, V. (1966) The Reactivating and Antidotal Actions of N,N'-Trimethylenebis (Pyridinium-4-Aldoxime) (TMB-4) and N,N'-Oxydimethylenebis (Pyridinium-4-Aldoxime) (Toxogonin) with Particular Reference to Their Effect on Phosphorylated Acetylcholinesterase in the Brain. Biochem. Pharmacol. 15, 1677-1690.
- Jakl, A. and Tabuch, J. (1971) Therapeutic Activity of Esterase-reactivators in Organophosphonates. Intoxication and Their Limits. Sb Ved Pr. Lek Fak. Karlovy Univ. Hrad 14, 153-163.
- Jensen-Holm, J., Lausen, H.H., Milthers, K. and Möller, K.O. (1959) Determination of the Cholinesterase Activity in Blood and Organs by Automatic Titration. With some Observations on Serious Errors in the Method and Remarks of the Photometric Determination. Acta. Pharmacol. et Toxicol. 15, 384-394.
- Johnson, D.D. and Stewart, W.C. (1970) The Effects of Atropine, Pralidoxime and Lidocaine on Nerve-Muscle Activity and Respiratory Function in Organophosphate-Treated Rabbits. Can. J. Physiol. Pharmacol. 48, 625-630.
- Karlog, O., Nimb, M. and Poulson, E. (1958) Parathion Poisoning Treated With Picoline-2-Aldoxime Iodide. Ugeskrift Laeger 129, 177-183.
- Kewitz, H. and Nachmansohn, D. (1957) A Specific Antidote Against Lethal Alkyl Phosphate Intoxication. IV. Effects in Brain. Arch. Biochem. Biophys. 66, 271-283.
- Mayer, O. and Michalek, H. (1971) Effects of DFP and Obidoxime on Brain Acetylcholine Levels and on Brain and Peripheral Cholinesterases. Biochem. Pharmacol. 20, 3029-3032.
- Milosevic, M.P. and Andjelhovic, D. (1966) Reactivation of Paraoxon-Inactivated Cholinesterase in the Rat Cerebral Cortex by Pralidoxime Chloride. Nature 210, 206.
- Milosevic, M., Terzic, M. and Vojvodić, V. (1961) Protection Against Lethal Phosphamidone Poisoning by nn-Trimethylene Bis(4-hydroxyiminomethyl-Pyridinium Bromide) (TMB-4) Arch. Int. Pharmacodyn. Therap. 132, 180-188.

REFERENCES (Con't)

- Namba, T. and Hiraki, K. (1958) PAM (Pyridine-2-Aldoxime Methiodide) Therapy for Alkyl Phosphate Poisoning. *J. Am. Med. Assoc.* 166, 1834-1839.
- Natoff, I.L. and Reiff, B.B. (1970) Quantitative Studies of the Effect of Antagonists on the Acute Toxicity of Organophosphates in Rats. *Brit. J. Pharmacol.* 40, 124-134.
- O'Leary, J.F., Harrison, B., Groblewski, G. and Wills, J.H. (1959) The Effects of 2-Formyl-1 Methylpyridinium Iodide Oxime (2-PAM) on Re-activation of Tissue Cholinesterase Following Poisoning by a Certain Phosphate Anticholinesterase. *Fed. Proc.* 18, 430.
- Rosenberg, P. (1960) In vivo Reactivation by PAM of Brain Cholinesterase Inhibited by Paraoxon. *Biochem. Pharmacol.* 3, 212-219.
- Schuchter, A., Kawel, H.G. and Schneider, J.A. (1960) Combined Treatment of Diethyl-p-Nitrophenyl Thiophosphate Poisoning with Pyridine-2-Aldoxime Methiodide and Atropine. *Arzneimittelforschung* 10, 399-400.
- Shek, E., Higuchi, T. and Bodor, N. (1976(a)) Improved Delivery Through Biological Membranes 2. Distribution, Excretion and Metabolism of N-Methyl-1,6-Dihydropyridine-2-Carbaldoxime Hydrochloride, a Pro-Drug of N-Methylpyridinium-2-Carbaldoxime Chloride. *J. Med. Chem.* 19, 108-112.
- Shek, E., Higuchi, T. and Bodor, N. (1976(b)) Improved Delivery Through Biological Membranes 3. Delivery of N-Methylpyridinium-2-Carbaldoxime Through the Blood-Brain Barrier in its Dihydropyridine Pro-Drug Form. *J. Med. Chem.* 19, 113-117.
- Wilson, C.W.M., Murray, A.W. and Titus, E. (1962) The Effects of Reserpine on Uptake of Epinephrine in Brain and Certain Areas Outside the Blood-Brain Barrier. *J. Pharmacol. Exp. Therap.* 135, 11-16.

UNCLASSIFIED

TABLE 1

EFFECT OF ROUTE OF ADMINISTRATION AND pH OF THE VEHICLE  
ON THE TOXICITY OF PRO-PAM AND PAM IN MICE

Compound	pH of vehicle	LD <sub>50</sub> (mg/kg)		
		IM	IV	IP
Pro-PAM	3.0	125 <sup>a</sup> (110-140)	168	173 (171-175)
	7.6	115		
PAM	3.0	210 (183-268)	95 (75-130)	162 (152-174)

a                      mean  
± 95% confidence limits

UNCLASSIFIED

UNCLASSIFIED

TABLE 2

COMPARISON OF THE EFFICACY OF PRO-PAM TO PAM  
IN PROTECTING MICE AGAINST ORGANOPHOSPHATE POISONING

Prophylaxis <sup>a</sup>	Dose (mg/kg)	Protective Ratio <sup>b</sup>		
		Soman	Sarin	DFP
PAM	50	0.96	1.28	1.41
Pro-PAM	50	0.87	1.31	2.20
PAM + Atropine	50 + 17.4	1.12	3.04	11.2
Pro-PAM + Atropine	50 + 17.4	1.23	3.21	19.2

<sup>a</sup> Prophylaxis was administered im in pH 3.0 citrate buffer  
10 minutes prior to receiving the organophosphate sc.

<sup>b</sup> Protective Ratio = 
$$\frac{\text{LD}_{50} \text{ of prophylaxis and organophosphate}}{\text{LD}_{50} \text{ of organophosphate}}$$

UNCLASSIFIED



UNCLASSIFIED

TABLE 3

COMPARISON OF THE EFFICACY OF PRO-PAM TO PAM  
IN PROTECTING GUINEA PIGS AGAINST ORGANOPHOSPHATE POISONING

Prophylaxis <sup>a</sup>	Dose (mg/kg)	Protective Ratio	
		Soman	Sarin
Pro-PAM + Atropine	15 + 17.4	2.7	> 34 <sup>b</sup>
PAM + Atropine	15 + 17.4	1.8	> 34

<sup>a</sup> The prophylaxis was administered im in pH 3.0 citrate buffer 10 minutes prior to sc administration of the organophosphate.

<sup>b</sup> Two of 10 animals died, whereas none died of those receiving PAM + atropine.

UNCLASSIFIED

UNCLASSIFIED

TABLE 4

COMPARISON OF PRO-PAM AND PAM AS COMPONENTS  
OF PROPHYLAXIS AND THERAPY REGIMEN OF SOMAN POISONING  
IN FEMALE GUINEA PIGS

Compound	Mortality	
	24 hour	48 hour
Pro-PAM <sup>a</sup>	3/5	4/5
PAM C1	0/4	1/4

<sup>a</sup> either PAM C1 (15 mg/kg) or Pro-PAM (15 mg/kg) + pyridostigmine (100 µg/kg) were administered im 30 minutes prior to receiving 5 LD<sub>50</sub>s of soman sc. One minute later atropine (17.4 mg/kg) + either PAM C1 (15 mg/kg) or Pro-PAM (15 mg/kg) was administered im.

UNCLASSIFIED

UNCLASSIFIED

TABLE 5

EFFECTIVENESS OF PRO-PAM IN REACTIVATING  
ORGANOPHOSPHATE-INHIBITED MOUSE BRAIN AChE

Prophylaxis	$\mu\text{mol ACh hydrolyzed/g/min}^a$		
	sarin	soman	DFP
PAM + Atropine <sup>b</sup>	$0.09 \pm 0.02^c$ (8)	$1.47 \pm 0.15$ (15)	$0.21 \pm 0.03$ (5)
Pro-PAM + Atropine	$0.60 \pm 0.06$ (8)	$2.13 \pm 0.32$ (10)	$0.69 \pm 0.18$ (4)

<sup>a</sup> Control  $5.09 \pm 0.7$   
(6)

<sup>b</sup> Atropine (17.4 mg/kg) + oxime (50 mg/kg) were administered im 10 minutes prior to sc administration of either sarin (300  $\mu\text{g/kg}$ ), soman (130  $\mu\text{g/kg}$ ) or DFP (25  $\mu\text{g/kg}$ ).

<sup>c</sup> mean  $\pm$  SEM  
(N)

UNCLASSIFIED

UNCLASSIFIED

TABLE 6

EFFECTIVENESS OF PRO-PAM IN REACTIVATING  
ORGANOPHOSPHATE-INHIBITED GUINEA PIG BRAIN AChE

Prophylaxis	$\mu\text{mol ACh hydrolysed/g/min}^a$	
	sarin	soman
PAM + Atropine <sup>b</sup>	$0.26 \pm 0.06^c$ (5)	$1.30 \pm 0.11$ (5)
Pro-PAM + Atropine	$0.44 \pm 0.04$ (5)	$0.69 \pm 0.05$ (5)

<sup>a</sup> Control  $5.22 \pm 0.13 \mu\text{mol ACh hydrolysed/g/min}$  N=5

<sup>b</sup> Atropine (17.4 mg/kg) + oxime (15 mg/kg) were administered im 10 minutes prior to sc administration of sarin (700  $\mu\text{g/kg}$ ) or soman (25  $\mu\text{g/kg}$ ).

<sup>c</sup> mean  $\pm$  SEM  
(N)

UNCLASSIFIED

UNCLASSIFIED

TABLE 7

BRAIN AChE ACTIVITY IN MICE EXPOSED TO SARIN<sup>a</sup>

Pretreatment	Dose (mg/kg)	Dose of Sarin ( $\mu$ g/kg)	Status	$\mu$ mol ACh/g <sub>b</sub> tissue/min	N
---	---	125	dead	$0.84 \pm 0.11$	8
---	---	125	alive	$0.46 \pm 0.37$	2
atropine	17.4	125	dead	$0.37 \pm 0.08$	4
atropine + PAM Cl	17.4 50.0	125	alive	$0.09 \pm 0.02$	8
---	---	300	dead	0	5

<sup>a</sup> Mice received sarin (125  $\mu$ g/kg) sc. AChE levels in 1 hour survivors and those dead were measured.

<sup>b</sup> Control AChE activity  $5.09 \pm 0.7$  (N=6)  $\mu$ mol ACh/g tissue/min.

UNCLASSIFIED

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D	
(Security classification of title, body of abstract and indexing annotation must be entered when the overall document is classified)	
1. ORIGINATING ACTIVITY DEFENCE RESEARCH ESTABLISHMENT SUFFIELD	2a. DOCUMENT SECURITY CLASSIFICATION UNCLASSIFIED
	2b. GROUP
3. DOCUMENT TITLE EFFICACY OF PRO-PAM (N-METHYL-1,6-DIHYDROPYRIDINE-2-CARBALDOXIME HYDROCHLORIDE) AS A TREATMENT FOR ORGANOPHOSPHATE POISONING	
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)	
5. AUTHOR(S) (Last name, first name, middle initial) Clement, J.G. (10) J.G./Clement (12)	
6. DOCUMENT DATE (11) Feb 1978	7a. TOTAL NO. OF PAGES 78 23p (12)
7b. NO. OF REFS 29	8a. PROJECT OR GRANT NO. 13D16
8b. CONTRACT NO.	9a. ORIGINATOR'S DOCUMENT NUMBER(S) SUFFIELD TECHNICAL PAPER NO. 487 DRES-TECHNICAL PAPER-487 (14)
	9b. OTHER DOCUMENT NO.(S) (Any other numbers that may be assigned this document) =
10. DISTRIBUTION STATEMENT "UNLIMITED DISTRIBUTION"	
11. SUPPLEMENTARY NOTES	12. SPONSORING ACTIVITY
13. ABSTRACT The efficacy of Pro-PAM (N-methyl-1,6-dihydropyridine-2-carbaldoxime hydrochloride) was compared to pyridine-2-aldoxime chloride (PAM) as a treatment for organophosphate poisoning in mice and guinea pigs. Pro-PAM was generally less toxic than PAM in mice and pH of the vehicle did not appear to alter the toxicity of Pro-PAM. Pro-PAM alone and combined with atropine improved the protective ratio (PR) of DFP and sarin in mice, whereas it improved very slightly the PR of soman in guinea pigs. The PR obtained with Pro-PAM combined with carbamate prophylaxis and atropine therapy versus soman poisoning was inferior to that obtained with PAM. In most cases Pro-PAM and atropine prophylaxis produced higher brain acetylcholinesterase (AChE) levels than PAM and atropine, however there was no correlation between brain AChE levels and survival. The significance of this lack of correlation is discussed. Pro-PAM was not a significant improvement over PAM with regards to therapy of organophosphorus poisoning. (U)	

403 104 -

elf

## KEY WORDS

Sarin  
Soman  
Pro-PAM  
Oxime

## INSTRUCTIONS

1. **ORIGINATING ACTIVITY** Enter the name and address of the organization issuing the document.
- 2a. **DOCUMENT SECURITY CLASSIFICATION:** Enter the overall security classification of the document including special warning terms whenever applicable.
- 2b. **GROUP** Enter security reclassification group number. The three groups are defined in Appendix 'M' of the DRB Security Regulations.
3. **DOCUMENT TITLE** Enter the complete document title in all capital letters. Titles in all cases should be unclassified. If a sufficiently descriptive title cannot be selected without classification, show title classification with the usual one-capital-letter abbreviation in parentheses immediately following the title.
4. **DESCRIPTIVE NOTES** Enter the category of document, e.g. technical report, technical note or technical letter. If appropriate, enter the type of document, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.
5. **AUTHOR(S)** Enter the name(s) of author(s) as shown on or in the document. Enter last name, first name, middle initial. If military, show rank. The name of the principal author is an absolute minimum requirement.
6. **DOCUMENT DATE** Enter the date (month, year) of Establishment approval for publication of the document.
- 7a. **TOTAL NUMBER OF PAGES:** The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.
- 7b. **NUMBER OF REFERENCES:** Enter the total number of references cited in the document.
- 8a. **PROJECT OR GRANT NUMBER:** If appropriate, enter the applicable research and development project or grant number under which the document was written.
- 8b. **CONTRACT NUMBER** If appropriate, enter the applicable number under which the document was written.
- 9a. **ORIGINATOR'S DOCUMENT NUMBER(S)** Enter the official document number by which the document will be identified and controlled by the originating activity. This number must be unique to this document.
- 9b. **OTHER DOCUMENT NUMBER(S)** If the document has been assigned any other document numbers (either by the originator or by the sponsor), also enter this number(s).
10. **DISTRIBUTION STATEMENT:** Enter any limitations on further dissemination of the document, other than those imposed by security classification, using standard statements such as:
  - (1) "Qualified requesters may obtain copies of this document from their defence documentation center."
  - (2) "Announcement and dissemination of this document is not authorized without prior approval from originating activity."
11. **SUPPLEMENTARY NOTES.** Use for additional explanatory notes.
12. **SPONSORING ACTIVITY:** Enter the name of the departmental project office or laboratory sponsoring the research and development. Include address.
13. **ABSTRACT:** Enter an abstract giving a brief and factual summary of the document, even though it may also appear elsewhere in the body of the document itself. It is highly desirable that the abstract of classified documents be unclassified. Each paragraph of the abstract shall end with an indication of the security classification of the information in the paragraph (unless the document itself is unclassified) represented as (TS), (S), (C), (R), or (U).  
  
The length of the abstract should be limited to 20 single-spaced standard typewritten lines; 7 1/4 inches long.
14. **KEY WORDS:** Key words are technically meaningful terms or short phrases that characterize a document and could be helpful in cataloging the document. Key words should be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context.